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A child with dyserythropoietic anemia and megakaryocyte dysplasia due to a novel 5'UTR *GATA1*s splice mutation

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Abstract

We describe a child with dyserythropoietic anemia; thrombocytosis; functional platelet defect and megakaryocyte dysplasia. We show that (i) this constellation of hematopoietic abnormalities was due to a germline mutation within the 5'UTR of *GATA1*; (ii) the mutation impaired a 5'UTR *GATA1* splicing site, promoting production of the shortened *GATA1*s isoform lacking the N-terminus; (iii) expression of the *GATA1* N-terminus is restricted to erythroblasts and megakaryocytes in normal marrow, consistent with the patient's abnormal erythropoiesis and megakaryopoiesis. Our findings provide insights into the clinically relevant *in vivo* function of the N-terminal domain of *GATA1* in human hematopoiesis.

Keywords

Dyserythropoietic anemia; megakaryocyte dysplasia; *GATA1*

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Authorship

G.N. and J.Z. directed patient's diagnostics and clinical management; J.Z. designed and performed Western blotting and RT-PCR experiments; C.T. and M.C. designed, performed and analyzed immunohistochemistry experiments; G.N. acquired microscopy images and analyzed data; G.N. wrote and all authors edited the paper.

Conflict of interest disclosure

The authors declare no competing financial interests.

Introduction

Germline mutations within the X-linked transcription factor *GATA1* occur in a spectrum of childhood blood disorders (reviewed in [1]). Alternative splicing produces two *GATA1* isoforms [2]: full-length *GATA1* (fl*GATA1*) and shortened *GATA1* (*GATA1s*), which lacks the N-terminal domain.

Rare defects known as “*GATA1s* mutations” [3] reduce synthesis of full-length *GATA1* while *GATA1s* is still produced. Acquired *GATA1s* mutations drive transient myeloproliferative disorder and AML in Down syndrome patients (reviewed in [4–6]). Germline *GATA1s* mutations in individuals without trisomy 21 have been associated with Diamond-Blackfan anemia (DBA) [7,8], a syndrome of RBC aplasia and otherwise normal hematopoiesis [9]. DBA-associated ribosomopathy impairs *GATA1* synthesis [10], perhaps because the complex structure of the *GATA1* 5′UTR demands error-free ribosomes for efficient translation. Thus, ribosomopathy has been mechanistically linked to *GATA1* and erythropoiesis failure in DBA. However, thrombocytopenia with structural platelet abnormalities and dyserythropoiesis [11] as well as childhood myelodysplastic syndrome (MDS) [12] were reported in other *GATA1s* families. Since *GATA1s* patients are rare and clinically heterogeneous, the clinical impact of inherited *GATA1s* mutations on human hematopoiesis remains to be fully elucidated.

Results

We describe a child with dyserythropoietic anemia, megakaryocyte dysplasia and platelet malfunction due to a disease-causing mutation within the 5′UTR of *GATA1*. We explored the impact of this novel mutation on the expression of *GATA1* splice variants in human bone marrow. Our findings support the role for the N-terminus of *GATA1* in *in vivo* megakaryocyte function and erythropoiesis.

A 4-year old male developed fatigue and pallor secondary to anemia (hemoglobin: 4.4 g/dL). His growth and development were normal with no congenital malformations. His past blood counts revealed progressive macrocytic anemia with reticulocytopenia and persistent fetal hemoglobin first noted at 3 months of age, as well as chronic thrombocytosis (platelets: 387-947,000/mm³) and occasional neutropenia with no frequent infections (lowest absolute neutrophil count: 495/mm³) (Figure 1A). The folate and vitamin B12 levels were normal. Normal DEB chromosome-breakage test and lymphocyte telomere length excluded Fanconi anemia and dyskeratosis congenita, respectively.

DBA was initially suspected due to progressive macrocytic anemia beginning in infancy [9]. However, the patient’s bone marrow analysis revealed not only paucity of RBC precursors (Figure 1B–C) and dyserythropoiesis (Supplementary Figure 1), but also prominent megakaryocytosis (Figure 1B–C) with megakaryocyte dysplasia (Figure 1B; Supplementary Figure 2), which is not seen in classic DBA [9]. Accordingly, sequencing of the patient’s nine DBA-associated ribosome genes (*RPL11*, *RPL35a*, *RPL5*, *RPS10*, *RPS17*, *RPS19*, *RPS24*, *RPS26*, and *RPS7*) and deletion-duplication analysis of *RPS19*, *RPL5*, *RPL11*, *RPL35A*, *RPS17* and *RPS26* produced normal results. Normal cytogenetics, MDS-FISH and

blast count excluded MDS. Thus, we asked whether his anemia mimicking DBA [7,8] but associated with megakaryocyte dysplasia [11] reflected a germline *GATA1* defect. Indeed, Sanger sequencing revealed a novel mutation within the 5'UTR of *GATA1* (*c.-21A>G* or *c.-19-2A>G*) at position 48,791,089 on the X-chromosome (GRCh38.p2 primary assembly), which affects the absolutely conserved *A* in the -2 position of the splice acceptor site (Figure 1D). Consistent with an X-linked recessive inheritance, mother was an asymptomatic carrier, and all healthy male siblings had wild-type *GATA1* (Figure 1D).

We hypothesized the *GATA1^{c.-21A>G}* transcript splicing is abnormal as our *in silico* analysis [13] suggested disruption of a 5'UTR consensus splice site. Accordingly, the *GATA1^{c.-21A>G}* mutation decreased *in vivo* flGATA1 expression (Figure 1E–F), which is consistent with the *GATA1s* phenotype [7,8,11,12]. The patient's anemia improved on corticosteroids similar to other *GATA1s* individuals [7,8,12]. Thus, the *GATA1^{c.-21A>G}* mutation produced the *GATA1s* phenotype through destruction of a splice site within the 5'UTR of *GATA1* (Figure 1G).

Past clinical reports have generated conflicting *in vivo* data regarding the impact of inherited *GATA1s* mutations on megakaryopoiesis in non-Down syndrome patients [7,8,11]. However, accumulation of dysplastic megakaryocytes in the *GATA1^{c.-21A>G}* marrow with peripheral thrombocytosis strongly suggests that flGATA1 regulates human megakaryocyte proliferation and function *in vivo* in individuals without trisomy 21. Moreover, the *GATA1^{c.-21A>G}* platelets displayed subclinical aggregation deficiencies (Supplementary Figure 3), further implicating flGATA1 in physiological megakaryopoiesis. Thus, we methodically examined *GATA1* splicing in human marrow, hypothesizing that flGATA1 expression correlates with the role of flGATA1 in distinct lineages. To that end, we developed an immunohistochemistry assay specific for the GATA1 N-terminus (Figure 2A). Full-length GATA1 was absent from the *GATA1^{c.-21A>G}* marrow, confirming that the mutation confers *GATA1s* phenotype (Figure 2B–C). Double-immunohistochemistry detected flGATA1 in healthy megakaryocytes and erythroblasts but not in other hematopoietic lineages (Figure 2C), consistent with the observation that loss of flGATA1 disrupted RBC and platelet production in our patient (Figure 1).

Discussion

GATA1 orchestrates the production of RBCs and platelets [14–16]. Alternative splicing provides an incompletely understood pathway to fine-tune this transcription factor's activity during hematopoiesis. Full-length GATA1 differs from the short isoform (*GATA1s*) by the presence of the 83 amino acid-long N-terminus, which activates GATA1-driven erythropoiesis [2,17] and recruits flGATA1 to a subset of megakaryocyte and erythroblast genes [3]. Thus, GATA1 isoforms control partially overlapping but not identical transcriptional modules of erythroblast and megakaryocyte maturation.

Acquired *GATA1s* mutations are key drivers of transient myeloproliferation and AML in Down syndrome [4–6]. Inherited *GATA1s* mutations that decrease production of flGATA1 through disrupting exon 2 splice sites [7,8,11] or the initiation codon [12] have been reported in rare non-Down syndrome patients (Figure 2). *GATA1s* patients uniformly

develop hyporegenerative anemia, confirming that the N-terminus of GATA1 is indispensable for erythropoiesis. However, since the reported clinical symptoms in *GATA1s* individuals range from DBA [7,8] to erythroblast and megakaryocyte dysplasia [11] and DBA progressing to MDS [12], the role of flGATA1 in other hematopoietic cell lines needs clarification.

Striking accumulation of dysplastic megakaryocytes in the *GATA1^{c.21A>G}* patient's marrow (Figure 1) suggests that loss of flGATA1 unleashes megakaryocyte proliferation *in vivo*, possibly due to de-repression of the E2F cell-cycle regulator [18]. In further support of this notion, independent *ex vivo* studies showed that hematopoiesis in *GATA1s* iPS cells is skewed towards generation of abnormal megakaryocytes at the cost of erythropoiesis [3]. Indeed, expression of the GATA1 N-terminus is restricted to erythroblasts and megakaryocytes (Figure 2), supporting a physiological role of flGATA1 in these hematopoietic lineages. This assay may provide a future screening tool for *GATA1s* phenotype. Intriguingly, both thrombocytopenia [11] and thrombocytosis (this work) occur in *GATA1s* individuals, including *GATA1s* patients diagnosed with DBA [8]. A systematic evaluation of megakaryopoiesis in additional *GATA1s* patients will determine whether megakaryocyte dysplasia is universally seen in *GATA1s* individuals. Potential genotype-phenotype correlations will remain unknown until more *GATA1s* patients are identified, especially since the same *GATA1^{c.220G>C}* mutation caused DBA-like phenotype in one family [8] and multilineage dysplasia in another [11].

The *GATA1^{c.21A>G}* platelets are defective (Supplementary Figure 3). This is in agreement with ultrastructural platelet abnormalities described in *GATA1^{c.220G>C}* family [11], likely secondary to disrupted cytoskeletal remodeling in flGATA1-deficient megakaryocytes [19]. Future studies will determine whether *GATA1s* patients develop clinically significant bleeding later in life.

Prominent megakaryocyte abnormalities (this work; [11]) may provide subtle clinical clues to differentiate *GATA1s* mutations from “classical” DBA caused by ribosomopathy, which is defined as pure red blood cell aplasia [9]. *GATA1* sequencing should be considered in males with congenital multi-lineage dysplasia and/or DBA-like clinical presentation. Interestingly, MDS-associated spliceosome mutations globally alter expression of multiple hematopoietic regulators, including *GATA1* [20]. Given the potential risk of MDS in *GATA1s* patients [12], hematopoiesis should be closely monitored in individuals with inborn *GATA1s* mutations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations key

CD	Cluster of differentiation
DEB	Diepoxybutane
DBA	Diamond-Blackfan anemia
FISH	Fluorescent in situ hybridization
flGATA1	Full-length GATA1
GATA1	Globin transcription factor 1
GATA1s	Shortened GATA1
iPS cells	Induced pluripotent stem cells
IRB	Institutional Review Board
MDS	Myelodysplastic syndrome
NIH	National Institutes of Health
RBC	Red blood cell(s)
RNA	Ribonucleic acid
RPL	Ribosomal protein L
RPS	Ribosomal protein S
RT-PCR	Reverse transcription-polymerase chain reaction
5'UTR	5' untranslated region

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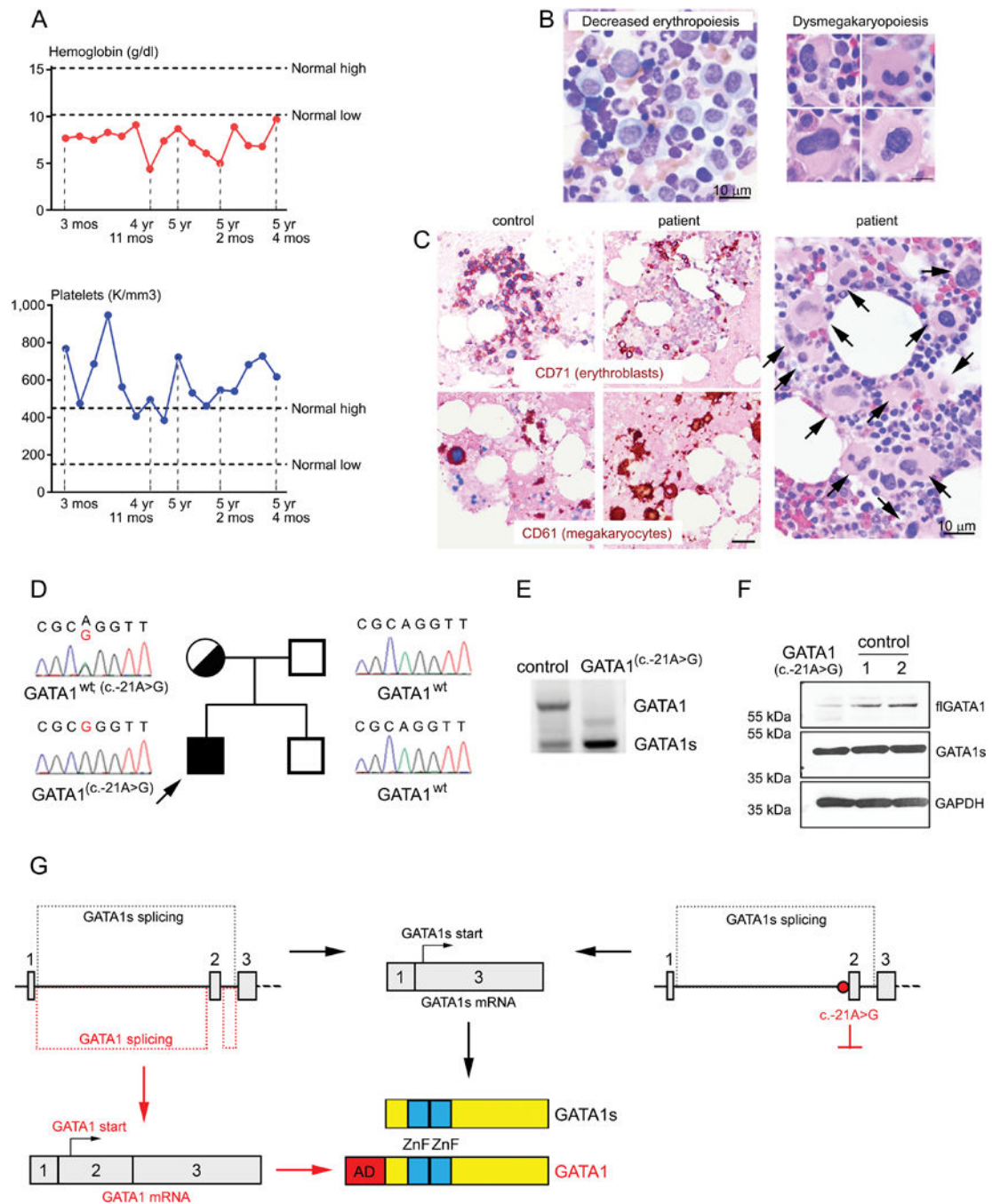


Figure 1. Hypoplastic anemia, megakaryocyte dysplasia and thrombocytosis due to mutation within the 5' UTR of GATA1

(A) Chronic anemia and thrombocytosis over the course of 5 years. (B) Decreased erythropoiesis and megakaryocyte dysplasia seen on bone marrow aspirate. (C) Immunohistochemistry with CD71 (erythroblast marker) and CD61 (megakaryocyte marker) reveals decreased erythropoiesis and accumulation of megakaryocytes in the patient's bone marrow compared to a healthy individual. Right panel shows dysplastic megakaryocytes (black arrows) in the patient's marrow (Wright-Giemsa stain). (D) *GATA1* sequencing reveals a novel mutation in the affected child. RT-PCR and Western blotting demonstrate

decreased full-length GATA1 transcript (E) and protein (F) in the patient. (G) Schematic representation of GATA1 alternative splicing (only first three exons are shown for simplicity). The *GATA1^{c-.21A>G}* mutation produces *GATA1s* phenotype by disrupting full-length GATA1 splicing.

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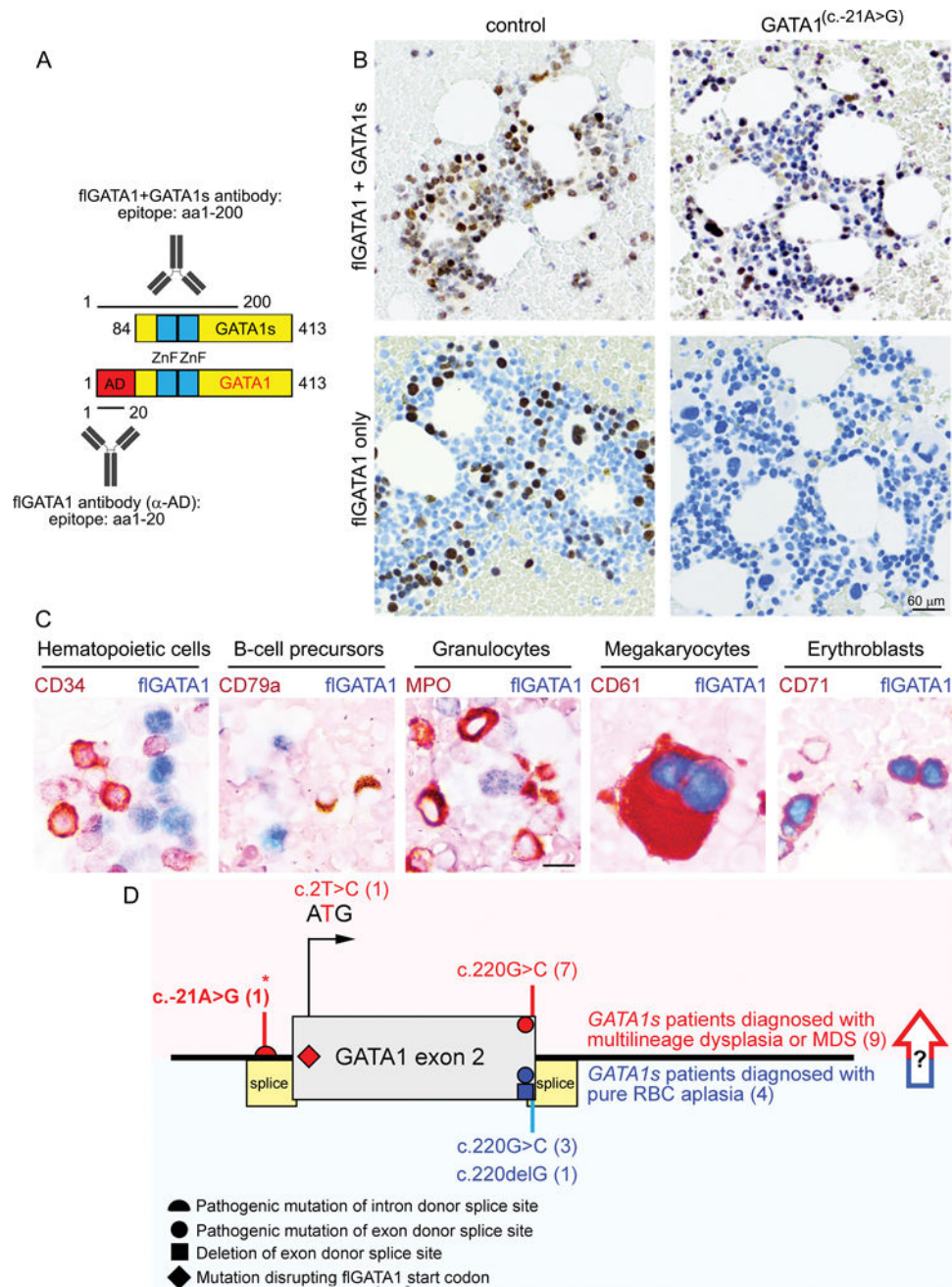


Figure 2. Full-length GATA1 expression is restricted to erythrocyte and megakaryocyte precursors during hematopoiesis

(A) Antibodies used for immunohistochemistry. Antibody against the N-terminus of GATA1 recognizes only full-length GATA1, while the C-terminal antibody recognizes both GATA1 isoforms (flGATA1 and GATA1s). (B) Loss of flGATA1 expression in the *GATA1*^{c.-21A>G} patient's bone marrow. Note that (i) flGATA1 is not expressed in all hematopoietic cells of a healthy individual, and (ii) GATA1s production is not affected by the *GATA1*^{c.-21A>G} mutation. (C) Expression of N-terminal GATA1 domain (flGATA1; blue) is restricted to erythroblasts and megakaryocytes during human hematopoiesis. Appropriate hematopoietic

lineage markers (brown) were co-stained as shown. (D) *GATA1s* mutations cause a range of phenotypes from Diamond-Blackfan anemia (red) to multilineage hematopoietic dysplasia (blue). Novel mutation described in this work is marked with asterisk. The arrow indicates that the long-term risk of MDS in *GATA1s* patients presenting with pure RBC aplasia remains to be determined as one *GATA1s* patient initially diagnosed with DBA developed MDS later in childhood [12].

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